UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

005516

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SEP 26 1986

MEMORANDUM

SUBJECT: Evaluate Review of Feeding and Oncogenicity Study in the Rat for Dicamba (Dynamac Corporation)

FROM: Stephanie April, Ph.D.
Section III, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Robert J. Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Marcia Van Gemert, Ph.D. M. Van Gemert 9.18.86
Head, Section III
Toxicology Branch
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and

Theodore Farber, Ph.D., Chief Theodore M. Farber 9.15.86
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Citation: Goldenthal, E.I.; Geil, R.G.
Lifetime Dietary Study in Rats -
(Unpublished Study No. 163-694 prepared
for Velsicol Chemical Corporation,
Chicago, IL, Dated February 28, 1985)
Accession No. 258115-258121.

Conclusion:

Dicamba was not found to be oncogenic in rats under the conditions of this study. The oncogenic and systemic NOEL = > 2500 ppm (HDT). This study satisfies the requirement for one rodent chronic and oncogenicity study.

17840

Classification: Core minimum.

Data Gaps:

Second chronic oncogenicity in rodent; chronic in nonrodent.

Summary and Discussion:

Attached is a review of the above-mentioned study completed as a contract with Dynamac. I have reviewed this study and edited this document submitted to EPA by Dynamac. The Toxicology Branch is in concurrence with the presently submitted and amended Dynamac document.

The only problem that requires comment is that the doses given did not elicit any significant toxic response. The doses used (50, 250, or 2500 ppm) in the diet for 115 weeks in the males and 117 weeks in the females were selected by Velsicol after conferring with the Agency on the basis of the results from other long-term studies with dicamba. Higher doses of dicamba in either rats or mice resulted in excessively high mortalities in previous chronic studies precluding the completion of adequate chronic testing of dicamba.

In this study, dicamba was fed to male Charles River CD rats for 115 weeks and to female Charles River CD rats for 117 weeks at the above-cited doses. No clinical signs of toxicity, or effects on survival, mean body weights or weight gains, food consumption, hematology, clinical chemistry, or urinanalysis that were related to administration of dicamba were reported. The macroscopic and histopathological findings in test animals were similar to that in the control animals.

Attachment

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EPA: 68-02-4225
DYNAMAC No. 047-A1-7
December 31, 1985

DATA EVALUATION RECORD

DICAMBA (Technical)

Chronic Toxicity and Oncogenicity Study in Rats

STUDY IDENTIFICATION: Goldenthal, E. I. and Geil, R. G. Lifetime dietary toxicity and oncogenicity study in rats. (Unpublished study No. 163-694 prepared by International Research and Development Corporation, Mattawan, MI, for Velsicol Chemical Corporation, Chicago, IL; dated February 28, 1985.) Accession No. 258115-258121.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I Cecil Felkner

Date: 12-31-85

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1. CHEMICAL: Dicamba.

2. TEST MATERIAL: Dicamba, Technical Reference Standard, lot No. 5262110, was described as a light tan solid with a purity of 86.8 percent.

3. STUDY/ACTION TYPE: Chronic toxicity/oncogenicity study in rats.

4. STUDY IDENTIFICATION: Goldenthal, E. I. and Geil, R. G. Lifetime dietary toxicity and oncogenicity study in rats. (Unpublished study No. 163-694 prepared by International Research and Development Corporation, Mattawan, MI, for Velsicol Chemical Corporation, Chicago, IL; dated February 28, 1985.) Accession No. 258115-258121

5. REVIEWED BY:

J. Fielding Douglas, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: James R Plaut for JFD
Date: December 31, 1985

William McLellan, Ph.D.
Independent Reviewer
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Signature: I. Cecil Felkner for
Date: 12-31-85

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
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Date: 12-31-85

Stephanie April, Ph.D.
EPA Reviewer

Signature: Stephanie P April
Date: 1/2/86

Clint Skinner, Ph.D.
EPA Section Head

Signature:
Date: 1-10-86

7. CONCLUSIONS:

A. Under the conditions of the study, Dicamba was not oncogenic when fed to Charles River CD rats for 115 weeks to males or 117 weeks to females at dietary levels of 50, 250, or 2,500 ppm. There were no effects of dosing on clinical signs of toxicity, survival, mean body weights or weight gains, food consumption, and hematologic, clinical chemistry, or urinary parameters. Organ weights, macroscopic findings, and nonneoplastic histologic findings were similar among dosed and control groups. The NOEL is 2,500 ppm Dicamba, the highest dose tested. Since an effect level was not achieved, it is possible that the animals might have tolerated a higher dose.

B. The study is Core Minimum for oncogenicity; the highest dose tested was tolerated without any adverse effects.

Item 8--see footnote 1.

9. BACKGROUND:

In a previous 90-day study, conducted by International Research and Development Corporation in 1980, a LOEL was established at 10,000 ppm Dicamba in the diet based on body weights, food consumption, and vacuolization in the liver and the NOEL was established at 5,000 ppm. The NOEL in a 4-week pilot study using levels up to 30,000 ppm was also 5,000 ppm. A high dose of 10,000 ppm in a mouse chronic study (one-half the NOEL of a 4-week pilot study) caused excessive mortality in an unacceptable study. To avoid possible excessive mortality, a high dose for this rat study was set at 2,500 ppm Dicamba.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. The test material, technical Dicamba, lot No. 52625110, had a stated purity of 86.8 percent and was described as a light tan solid.

2. CD rats (240 of each sex), obtained from Charles River Breeding Laboratories, were randomized into four groups of 60/sex and caged individually in a temperature- and humidity controlled animal room with a 12-hour light/dark cycle. Food

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[1] Only items appropriate to this DER have been included.

(Purina Certified Chow #5002) and water were available ad libitum. The animals were approximately 5 weeks old at initiation of dosing, and males weighed approximately 210 g and females approximately 165 g.

3. Groups of animals (60/sex) were fed diets containing Dicamba technical at levels of 0, 50, 250, or 2,500 ppm for 115 weeks for males or 117 weeks for females. Diets were prepared weekly, and analysis of test compound in diets was conducted at weeks 1-4, 8, and 13 and quarterly thereafter. Stability and homogeneity of test material in diets were tested prior to study initiation.

4. Animals were observed twice daily for toxic signs and mortality and once weekly for palpable masses. Body weights and food consumption were recorded weekly for the first 13 weeks and every 2 weeks thereafter.

5. Blood for hematology and clinical chemistry testing and urine for analysis were collected from 10 animals/sex/group at 6, 12, 18, and 24 months. Baseline determinations were made on 10 animals/sex on two occasions prior to study initiation. See Appendix A for the parameters measured.

6. An interim sacrifice was performed on 10 animals/sex/group at 12 months. All surviving animals were sacrificed by CO_2 asphyxiation; the males were sacrificed at 115 weeks and females at 117-118 weeks. The following organ weights were determined for animals at the 12-month and terminal sacrifice: liver, kidneys, heart, brain (with stem), testes, and ovaries (fixed). Complete gross and histopathologic examinations were performed on animals sacrificed by design as well as those found dead or sacrificed in extremis.

7. Statistical methods employed for body weights, food consumption, hematology, clinical chemistry, urinalysis, and absolute and relative organ weight data included Bartlett's test and analysis of variance. Treatment groups were compared to the same-sexed control group using t-statistics for either equal or unequal variances and Dunnett's multiple comparison tables. Survival data and time to neoplastic tumor development were analyzed using a computer program developed by Thomas et al.[2] The program includes procedures for survival analysis according to Kaplan-Meier, Cox's test for linear trends, and both Cox's test and Gehan Breslow's generalized Kruskal-Wallis test for survival distribution comparisons.

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[2] Thomas, D. G., Breslow, N., and Gart, J. J. Trend and homogeneity analysis of proportions and life table data. Computers and Biomedical Research 10(1977): 373-381.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

Dietary Analysis: The mean test compound content of all analyzed diets was 102±12, 103±11, and 104±7 percent of the target concentrations at nominal levels of 50, 250, and 2,500 ppm, respectively. During the first 52 weeks of the study, there were some wide variations between replicate samples. Reanalysis of some samples by the sponsor suggested that the variations were due to the methodology. Therefore, a revised method of analysis was used from week 52 to study termination. During this latter period, the ranges of analytical values for 50, 250, and 2,500 ppm diets were 99-105, 89-123, and 91-108 percent, respectively, whereas the ranges at the same doses for the entire study were 92-123, 83-128, and 91-114 percent of the nominal levels, respectively.

The test material was stable in the diets for 10 days at room temperature. Assays prior to study initiation indicated that when diets were mixed with the test compound for 30 minutes, homogeneity was acceptable. Analysis for homogeneity at week 39 indicated variability in the homogeneity of the 50 ppm diet; this was attributed to the analytical method but there was acceptable homogeneity in the 250-ppm and 2,500-ppm diets. The mean concentrations of analysis at the top, middle, and bottom samples (tested at week 39) were 102±45, 104±16, and 99±11 percent of the target level at 50, 250, and 2,500 ppm, respectively.

Clinical Observations and Mortality: Throughout the study the clinical observations indicated that there were no trends to suggest a compound-related effect. Findings common to dosed and control rats included red areas around the eyes, localized hair coat staining, localized hair loss, corneal opacity, and palpable masses. The incidence was similar in control and dosed groups. Mortality was similar among dosed and control groups; at 101 weeks survival ranged from 44-56 percent in male groups and 54-78 percent in female groups (Table 1).

Body Weights and Food Consumption: Significant ($p \leq 0.05$) increases in mean body weights in males receiving 2,500 ppm were seen at a few weekly intervals; however, mean body weights of dosed females did not differ significantly from controls. At termination, mean body weights in dosed males were 9.7-9.9 percent higher than in controls, mean body weights of females receiving 2,500 ppm were 11.2 percent lower than controls (Table 2).

Mean food consumption values were slightly, but significantly ($p \leq 0.05$), increased in males receiving 2,500 ppm at 9 of the 20 intervals measured during the first 27 weeks of the study; however, for the entire study food consumption was only 2 percent greater than the control mean value. Food consumption was similar in dosed and control females.

TABLE 1. Percent Survival at Selected Intervals in Rats Fed Dicamba (115 Weeks for Males, 117 Weeks for Females)[a]

	Percent Survival							
	Dietary Level (ppm)				Dietary Level (ppm)			
Week	0	50	250	2500	0	50	250	2500
	Males				Females			
53	98	96	96	100	98	98	100	98
79	88	82	78	90	90	88	88	92
101	56	52	52	44	78	64	54	76
115	22	24	38	30	46	34	30	42
117	--	--	--	--	44	30	28	40

[a]Based on 50 animals/sex/group, and does not include 10 animals/sex/group sacrificed after 52 weeks.

TABLE 2. Mean Body Weights at Selected Intervals for Rats Fed Dicamba (115 Weeks for Males, 117 Weeks for Females)[a]

Week	Mean Body Weights (g) at Dietary Levels (ppm) of 0	50	250	2500
Males				
1	213± 15.7	210± 15.2	207± 17.4	210± 16.4
13	507± 49.6	498± 49.0	496± 50.3	505± 47.5
29	612± 69.7	597± 76.3	597± 69.3	613± 65.9
51	722±102.5	706±106.6	695± 94.4	721± 97.0
79	745±127.8	751±111.6	730± 89.9	802±118.2
115	628±109.1	689±124.7	707±154.4	690±124.8
Females				
1	165± 13.8	165± 12.4	164± 13.1	165± 12.2
13	298± 28.7	297± 30.6	297± 25.0	299± 27.2
29	347± 41.4	347± 43.4	345± 38.3	351± 40.1
51	417± 70.9	415± 67.0	412± 64.4	421± 69.5
79	508±100.6	480± 84.1	477±124.8	495± 94.7
117	552±111.8	502±152.3	549±106.3	490± 99.1

[a]There were no significant differences ($p \geq 0.05$) in the selected values tabulated.

Hematology: There were no compound-related effects at any dietary level at any interval of analysis.

Clinical Chemistry: There were no toxicologically important effects of dosing on any clinical chemistry parameter. There was a significant ($p \leq 0.05$) increase in serum alkaline phosphatase in all dosed groups of males at the 18-month interval, but levels were reported within the normal range of the testing laboratory, and significant effects were not seen at other intervals (Table 3; see section 14). Cholesterol was significantly lower in males that received 50 ppm at the 6-month interval, but there were no dose-related trends or effects at other intervals (12, 18, or 24 months).

Urinalysis: None of the dosage levels showed a compound-related effect at any of the analysis intervals.

Organ Weights: There were no significant changes ($p > 0.05$) in mean absolute or relative organ weights for any dosage group at the 12-month interim sacrifice. There were no compound-related changes in mean absolute organ weights of either sex at study termination. A significant increase ($p < 0.05$) noted in kidney-to-body weight ratio of females receiving 2,500 ppm was considered to be random and unrelated to the test compound.

Gross Pathology: At the 12-month interim sacrifice, there were no compound-related macroscopic changes in rats of either sex. At termination of the study, there were occasional and spontaneous morphologic changes in the livers of dosed males and in the glandular stomachs and adrenal glands of dosed females. These occurred at higher frequencies in the dosed groups than in controls (Table 4), but these changes were not considered to be related to the test compound. There were no correlating histologic changes. Other gross findings were incidental and occurred at similar frequency in control and dosed groups.

Histopathology: Tables 5 summarizes neoplastic lesions reported at 12 months; and Table 6 summarizes lesions in animals that died, were sacrificed moribund, or sacrificed at termination. There was no evidence of an oncogenic effect. No statistical significance ($p \leq 0.05$) was found by pairwise comparison of dosed and control groups for any tumor or category of tumor. There was a significant ($p \leq 0.05$) linear trend (using the Cochran-Armitage trend test) for mixed malignant lymphomas and parafollicular carcinomas of the thyroid in males, but these were not considered toxicologically significant since there was no significance by pairwise comparison. Microscopic analysis of coronal sections of the head (10/group/sex) revealed one nasal papilloma, one nasal squamous carcinoma, and one oral papilloma in males receiving 50 ppm, one nasal polyp in a female receiving 50 ppm, and one oral papilloma in a male receiving 2,500 ppm. Examinations of additional coronal sections (data not found) did not indicate any oncogenic effect of dosing.

TABLE 3. Serum Alkaline Phosphatase Levels in Rats Fed Dicamba

Dietary Level (ppm)	Alkaline Phosphatase (IU/L)± SD at Month			
	6	12	18	24
0	44±10.5	46±10.9	32± 7.0	33±15.7
50	52±13.5	51±12.3	48±13.6**	37± 9.7
250	54±14.2	58±25.5	50±20.7*	77±75.4[b]
2,500	50±14.0	82±90.2[a]	49± 9.0**	42±13.4

[a]Omitting one outlier value gave 54±11.4 (reanalysis by our reviewers).

[b]Omitting one outlier value gave 53±15.5 (reanalysis by our reviewers).

*Significant difference at $p \leq 0.05$ level.

**Significant difference at $p \leq 0.01$ level.

TABLE 4. Frequent Gross Findings in Rats Fed Dicamba[a]

Organ/Finding	Males				Females			
	Dietary Level (ppm)				Dietary Level (ppm)			
	0	50	250	2500	0	50	250	2500
No. examined	49	49	48	50	49	49	50	49
Liver								
Discolored foci[b]	0	10	18	20	13	11	16	7
Masses	3	4	4	1	0	0	2	1
Adrenal								
Enlarged	2	1	2	1	4	7	9	10
Stomach, Glandular[c]								
Mucosal foci	5	4	4	4	1	7	5	5

[a]Includes animals sacrificed at termination and those that died or were sacrificed moribund from month 12 to termination.

[b]Red/black, trace/mild/moderate.

[c]Red/black/brown, trace/mild/moderate.

TABLE 5. Incidence of Neoplasms in Rats Fed Dicamba After 52 Weeks

	Males				Females			
	Dietary Level (ppm)				Dietary Level (ppm)			
Organ/Neoplasm	0	50	250	2500	0	50	250	2500
Adrenal	(11)[a]	(11)	(12)	(10)	(11)	(11)	(10)	(11)
cortical adenoma	0	1	0	0	1	0	0	2
Liver	(11)[a]	(11)	(12)	(10)	(11)	(11)	(10)	(11)
neoplastic nodules	0	0	0	1	0	0	1	0
Pituitary	(11)[a]	(11)	(12)	(10)	(11)	(11)	(10)	(11)
adenoma	0	2	1	2	1	2	4	2
Thyroid	(11)[a]	(11)	(12)	(10)	(11)	(11)	(10)	(11)
parafollicular carcinoma	0	0	1	0	0	0	0	0
Ovary					(11)[a]	(11)	(10)	(11)
granulosa cell tumor					0	0	0	1
Uterus					(11)[a]	(11)	(10)	(11)
polyp					0	2	0	0

[a] Number of tissues examined histologically; includes animals sacrificed by design (10/sex/group) and decedents.

TABLE 6. Summary of Neoplastic Lesions in Rats Fed Dicamba (115 Weeks for Males, 117 Weeks for Females)[a]

Organ/Neoplasm	Males Dietary Level (ppm) 0	50	250	2500	Females Dietary Level (ppm) 0	50	250	2500
Adrenal	(49)[b]	(49)	(48)	(50)	(49)	(49)	(50)	(49)
pheochromocytoma	14	9	12	14	1	4	3	5
adenoma	2	0	0	0	7	3	6	2
Brain	(49)[b]	(49)	(48)	(50)	(49)	(49)	(50)	(49)
astrocytoma	3	0	0	0	2	1	0	1
adenocarcinoma	0	0	0	0	0	3	0	0
Liver	(49)[b]	(49)	(48)	(50)	(49)	(49)	(50)	(49)
neoplastic nodule	10	1	8	2	2	5	2	2
carcinoma	2	4	2	1	0	0	2	0
nodule/carcinoma	12	5	10	3	2	5	4	2
Lymphoreticular[c]								
lymphoma, histiocytic	0	0	2	0	3	0	0	0
lymphoma, lymphocytic	0	0	2	0	3	0	2	0
lymphoma, mixed	0	0	0	4	0	0	0	0
lymphoma, total	0	9	4	4	6	0	2	0
granulocytic leukemia	0	0	0	0	2	0	0	1
Pancreas	(49)[b]	(48)	(48)	(50)	(49)	(49)	(49)	(49)
islet cell adenoma	4	2	2	1	3	2	1	3
adenomas	0	0	0	1	0	0	0	0
islet cell carcinoma	0	0	1	1	0	0	1	1
Pituitary	(49)[b]	(48)	(47)	(50)	(49)	(49)	(50)	(49)
adenoma	35	35	29	34	41	4	9	40
adenocarcinoma	1	0	0	1	0	3	0	0

[a]Includes animals at terminal sacrifice and those that died or were sacrificed between month 12 and termination. A neoplasm was not included in this tabulation if it only occurred in one animal in any group and could not be included with other tumors for statistical analysis.

[b]The numbers in parentheses are the number of tissues examined histologically.

[c]Number examined could not be determined; 49 or 50 animals/sex/group were examined histologically.

(Continued)

TABLE 6. Summary of Neoplastic Lesions in Rats Fed Dicamba (116 Weeks for Males, 117 Weeks for Females)[a] (Cont'd)

Organ/Neoplasm	Males Dietary Level (ppm) 0	50	250	2500	Females Dietary Level (ppm) 0	50	250	2500
Skin	(49)[b]	(49)	(48)	(50)	(49)	(48)	(50)	(49)
basal cell tumor	1	0	0	1	0	0	1	0
keratoacanthoma	3	0	2	1	1	0	1	0
papilloma	4	1	0	0	0	0	0	1
sebaceous adenoma	1	0	1	1	0	0	0	0
fibrosarcoma	2	0	0	0	0	0	0	0
squamous cell carcinoma	0	3	2	0	0	0	1	0
Testis	(49)[b]	(49)	(48)	(30)				
interstitial cell tumor	5	2	7	2				
Thyroid	(49)[b]	(49)	(48)	(50)	(49)	(49)	(50)	(49)
follicular adenoma	0	1	1	1	0	0	0	0
follicular carcinoma	0	1	0	0	0	0	0	0
parafollicular adenoma	2	5	5	3	5	1	3	6
parafollicular carcinoma	0	1	0	0	0	1	0	0
Mammary gland	(43)[b]	(42)	(45)	(44)	(49)	(49)	(50)	(48)
adenoma	0	0	0	0	0	1	3	0
fibroadenoma	0	0	1	0	24	23	20	23
adenocarcinoma	1	0	0	1	13	9	11	9
Ovary					(49)[b]	(48)	(49)	(49)
granulosa cell tumor (malignant)					1	2	0	0
Thymus	(45)[b]	(44)	(43)	(46)	(47)	(46)	(41)	(44)
thymoma	0	0	0	0	3	1	0	1
Uterus					(49)[b]	(49)	(50)	(49)
polyp					4	3	5	8

[a] Includes animals at terminal sacrifice and those that died or were sacrificed between month 12 and termination. A neoplasm was not included in this tabulation if it only occurred in one animal in any group and could not be included with other tumors for statistical analysis.

[b] The numbers in parentheses are the number of tissues examined histologically.

[c] Number examined could not be determined; 49 or 50 animals/sex/group were examined histologically.

(Concluded)

There were no nonneoplastic changes in rats of either sex that were considered related to dosing. There were occasional findings that were increased in a dosed group when compared to the control (Table 7), but these findings were considered incidental and normal variations for the strain of rat.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. There was no oncogenic effect of Dicamba when it was fed to male or female CD rats at dietary levels of 25, 250, or 2,500 ppm for up to 117 weeks. There were no signs of toxicity related to the test compound. Survival was comparable to control groups for males and females receiving 2,500 ppm; there was a slight increase in mortality from weeks 100-117 in females receiving 50 ppm and from weeks 82-117 in females receiving 250 ppm. There were no effects of dosing on body weights, food consumption, hematologic, biochemical and urinalysis determinations, organ weights, or macroscopic and microscopic findings. On the basis of the results of this lifetime study, the NOEL is considered to be 2,500 ppm, the highest dose tested.

B. A quality assurance statement was dated February 28, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Under the conditions of the study there was no oncogenic effect of Dicamba when fed to males or female CD rats at levels as high as 2,500 ppm for 115 to 117 weeks. It is our assessment that the rats may have tolerated a higher dose; however, there appears to be an adequate rationale for selecting the high dose (see section 9).

The design and conduct of the study were acceptable for assessing chronic toxicity and oncogenicity. There was a wide variation in replicate values for diet analysis during the first year of the study, particularly in the 50-ppm diet. However, we do not assess that this had any impact on the study since it was determined that there was a problem in methodology, and this problem was rectified by week 52. The variations in dietary analysis values were acceptable thereafter; for the overall study, the mean analytical levels were acceptably close to the intended levels. The impurities in the test material (13 percent) were not presented or discussed in the report.

Very few errors were found in the summary tables of the data, and the narrative and conclusions of the authors are supported by individual and summary data. In calculating means for some clinical chemistry parameters, outlying values were not omitted, which resulted in spuriously high values and standard deviations (e.g., Table 3). However, it is our assessment that the authors were correct in interpreting the biological significance of all data.

TABLE 7. Selected Nonneoplastic Histologic Changes in Rats Fed Dicamba (115 Weeks for Males, 117 Weeks for Females)

Sex	Organ/Lesion	Number Found at Dietary Level (ppm) 0	50	250	2500
Female					
	Brain	(49)[a]	(49)	(50)	(49)
	Ventricular dilation	15	18	20	30*,[b]
	Lymph node (abdominal)	(49)[a]	(49)	(49)	(49)
	Mild hyperplasia	10	5	13	17*,[b]
Male					
	Liver	(49)[a]	(49)	(48)	(50)
	Telangiectasis	11	11	19*	16
	Stomach (nonglandular)	(49)[a]	(49)	(48)	(50)
	Inflammation	3	4	8	8

[a]The numbers in parentheses are the numbers of tissues examined.

[b]Significant linear trend ($p < 0.05$) by the Cochran Armitage test; analysis by our reviewers.

*Significantly different from control incidence ($p \leq 0.05$); Fisher exact test, analysis by our reviewers.

The quality assurance statement indicated that the study was subjected to periodic inspections and review of the report. However, it did not state that there were any deviations from good laboratory practices that affected the quality or integrity of the study.

Histologic findings in this study agree well with the normal historical incidence of neoplastic and nonneoplastic findings in CD rats. Based on all finding in the study, the NOEL is 2500 ppm, the highest dose tested.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 1-4, 10-16, 19-23, 26-31.

APPENDIX A

Materials and Methods

005516

I. SUMMARY: Lifetime Dietary Toxicity and Oncogenicity Study in Rats
IRDC Study 163-694
Initiated July 1, 1981
Terminated October 3, 1983

Test article was offered in the diet to Charles River CD® rats at dosage levels of 50, 250 and 2500 ppm for up to 27 months. Summaries of the methods, results and conclusions are presented below.

A. EXPERIMENTAL DESIGN/METHODS

TEST ARTICLE

Identification: Technical Dicamba

Dietary Concentrations: 0.05, 0.25 and 2.50 g of Technical Dicamba per kg of prepared test diet.

Dietary Analysis: At IRDC prior to study initiation to determine stability and homogeneity of the test article in the diet and additionally on 4-2-82 due to a change in blending procedure.

Dietary Analysis: Conducted at IRDC; at time of preparation, weeks 1-4, 8, 13 and quarterly thereafter. Samples of each diet prepared weekly were frozen and stored at IRDC.

Test Article Samples: Sample sent to the Sponsor every 6 months.

TEST SYSTEM/IN-LIFE OBSERVATIONS

Animals: Charles River CD® rat; Charles River Breeding Laboratories, Inc., Portage, Michigan; approximately 5 weeks old at initiation.

Dosage Groups:

Dosage Level (ppm)	Number of Rats Male	Female
0 (Control)	60	60
50	60	60
250	60	60
2500	60	60

Randomization Procedure: Computerized random selection in a block design based on body weights. Animals with large absolute differences from the quarantine population body weight mean were eliminated prior to randomization and homogeneity of group body weight variances was used as the criteria for acceptance.

Housing/Environmental Conditions: Individually housed in hanging wire-mesh cages in an environmentally controlled room. Water and diet available *ad libitum*.

Diet: Purina® Certified Rodent Chow® #5002.

Observations: Overt toxicity, moribundity and mortality twice daily. Detailed observations including palpation for masses once weekly.

Measurements: Body weights and food consumption weekly for the first 13 weeks and once every 2 weeks thereafter.

CLINICAL PATHOLOGY

Baseline Determinations: 10 rats/sex/group on two occasions.

Study Determinations: 10 rats/sex/group; selected randomly prior to study initiation; at 6, 12, 18 and 24 months.

Hematology: Hematocrit value, hemoglobin concentration, erythrocyte count and MCH, MCV and MCHC (calculated), leukocyte count (total and differential), platelet count, reticulocyte count.

Biochemistry: Cholesterol, albumin, globulin (calculated), total protein, creatinine, electrolytes (sodium, potassium, chloride and calcium), phosphorus, lactic dehydrogenase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, glucose, urea nitrogen, total bilirubin.

Urinalysis: Volume, color and appearance, pH, specific gravity, protein, glucose, ketones, urobilinogen, nitrites, bilirubin, occult blood, microscopy of spun deposit.

PATHOLOGY

Unscheduled Necropsies: All animals dying or sacrificed in extremis.

Interim Necropsy: 12 months; 10 rats/sex/group; 6-30-82.

Terminal Necropsy: All surviving males on 9-12-83 and all surviving females at 27 months on 10-3-83.

Organ Weights: For rats sacrificed at 12 months or at termination: liver, kidney (2), heart, ovary (after fixation) [2], testis (2), brain (with stem).

Histopathology: On all animals; tissues fixed in formalin, sectioned at 5-8 microns, and stained with hematoxylin and eosin. Eyes fixed in glutaraldehyde.

Tissues Processed:

Adrenal (2), brain (3 levels: fore, mid and hind brain), eye with contiguous Harderian gland (2), esophagus, stomach, jejunum, colon, ovary (2), testis with epididymis (2), heart, kidney (2), liver (3 sections), lung with mainstem bronchi (all lobes), lymph nodes: mediastinal, mesenteric and regional when applicable, mammary region, pancreas, pituitary, prostate and seminal vesicle, salivary gland (mandibular with submandibular lymph node), sciatic nerve, skeletal muscle (thigh), skin, spinal cord (cervical), spinal cord and vertebrae (lumbar), spleen, sternum (bone and marrow), thymic region, thyroid - parathyroid complex, trachea, urinary bladder, uterus (2 horns and cervix), all tissue masses and all gross lesions.
In addition, 3 transverse sections through the head (includes tongue, nasal cavity, turbinates, paranasal sinuses, nasopharynx, portions of oral cavity and middle ear) were examined for all animals.

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STATISTICS

Methods: Body weights; food consumption; hematological, biochemical and urinalysis determinations and absolute and relative organ weights analyzed using Bartlett's test and analysis of variance. Treatment groups compared to the control group, by sex, using the appro-priate t-statistic (equal or unequal variance) and Dunnett's multiple comparison tables.
Survival data and data on time to neoplastic lesions analyzed using the computer program of Thomas, Breslow and Gart.

IV. TEST ARTICLE

A. RECEIPT AND DESCRIPTION

The test article was received from Velsicol Chemical Corporation, Chicago, Illinois, on May 28, 1981 as follows:

Label	Description
TECH. REF. STD. Dicamba 86.8% (GC) Lot 52625110	light tan solid (three containers received)

(Lids of containers read:)

Container 1: 10 kg
Container 2: 2.5 kg
Container 3: 2.5 kg

B. PREPARATION

1. Diet

Technical Dicamba was administered in the diet at dosage levels of 50, 250 and 2500 ppm.

The test article was ground with a mortar and pestle prior to weighing. Once weighed the test article was ground again with approximately 200 g of Certified Rodent Chow® #5002 using a mortar and pestle. The mixture was added to approximately 300 g of additional Certified Rodent Chow® #5002 and mixed for 5 minutes using a Hobart mixer. The resulting premix was added to a twin shell blender with additional Certified Rodent Chow® #5002 to yield 36 kg of prepared test diet. The blender was operated for 30 minutes with the intensifier bar operating during the entire blending procedure. Fresh diets were prepared weekly.

2. Samples

a. Test Article

A 5 gram sample of the test article was collected every 6 months and sent to the Sponsor for possible analysis.

b. Diet

Samples (approximately 100 g) of each control and test diet prepared were collected and stored frozen at IRDC. Samples of each control and test diet were collected and analysed at weeks 1-4, 8, 13, 26, 27, 39, 48, 52, 65, 78, 91, 104 and 117 for test article concentrations.

c. Homogeneity

Prior to study initiation samples were collected from the top, middle and bottom of diets blended for 10, 20 and 30 minutes and analysed for test article concentrations.

d. Stability

Prior to study initiation diet samples were collected and stored under normal laboratory conditions for up to 10 days and analysed for test article concentrations.

Mark W. Griggs 2/27/85

Mark W. Griggs, B.S. Date
Manager, Test Material Control

C. ANALYSIS

1. Analytical Method

a. Reagents

1. Diethyl ether
2. 10% H_2SO_4 - 10 ml conc. H_2SO_4 + 90 ml distilled water
3. 5% aqueous $NaHCO_3$
4. Conc. HCl
5. 1 N Methanolic HCl (8.3 ml concentrated HCl in 100 ml methanol)
6. Hexane
7. Ethereal diazomethane prepared from Diazald® (Aldrich Chemical Co.)

b. Extraction

1. A 10 g sample was extracted with 200 ml ether containing 0.5 ml of 10% H_2SO_4 in a blender for 5 minutes.
2. Extract was filtered under vacuum through Whatman #4 filter paper. The blender jar was washed with additional 50 ml ether and filter. Filtrates were combined.
3. Extract was placed in a 250 ml separatory funnel. Ether layer was extracted with 3 x 25 ml of 5% $NaHCO_3$. Aqueous layer was collected in an Erlenmeyer flask. Ether layer was discarded.
4. Aqueous phase was acidified to pH <1 with conc. HCl (requires 6 to 8 ml). Acidified aqueous phase was transferred to same separatory funnel and partitioned with 3 x 50 ml ether.
5. Combined ether extracts were collected in either a 50 ml volumetric flask (Groups 1 & 2) or a 250 ml volumetric flask (Groups 3 & 4) and brought to volume with diethyl ether. One (1.0) ml of the well mixed extract of Group 4 was diluted to 10 ml with ether to yield a theoretical concentration of 10 ug test article/ml. Extracts of Groups 1, 2 & 3 containing a theoretical concentration of 10 ug/ml were ready for Step 1c as is.

c. Methylation

1. A 1.0 ml aliquot was transferred from the 10 ug test article/ml extracts to a 250 ml flat bottom flask. Ten (10) to 15 ml of ethereal diazomethane and 3 drops of methanolic HCl was added. The solutions were mixed well and allowed to stand overnight in a hood.

u - Read as micro (10^{-6})

2. One (1) ml of toluene was added and the extract was concentrated to 1 ml using a rotary vacuum evaporator.

3. The residue was transferred to a 200 ml volumetric flask with multiple hexane rinses and brought to volume with hexane.

d. Preparation of Standard

1. About 10 mg of the test article was accurately weighed and dissolved in 100 ml of toluene. A 1.0 ml aliquot was diluted to 10 ml with toluene to yield a 10 ug/ml standard solution (working standard).

2. A 1.0 ml aliquot of the working standard was methylated concurrently with sample extracts as described in 1, c, 1-3.

e. Gas Chromatographic Analysis

Instrument - Varian 2400 or equivalent equipped with ^{63}Ni electron capture detector.

Column - 6' x 4 mm glass column packed with 10% OV-17 on Gas Chrom Q.

Temperatures - Oven - 150°C
Detector - 280°C
Injector - 285°C

Flow rate (N_2) - Carrier 38 ml/min

f. Calculations

$$\frac{\text{Peak Height Sample}}{\text{Peak Height Standard}} \times \text{concentration of standard (ug/ml)} \times$$

$$\text{Dilution factor} \times \frac{1}{\text{Sample Weight (g)}} = \text{ppm test article found}$$

u - Read as micro (10^{-6})

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The revised analytical method described below was used beginning at study week 52 dietary analyses.

a. Apparatus

1. Polytron homogenizer
2. Buchner funnels
3. Whatman #4 filter paper to fit Buchner funnel
4. Diazomethane generating kit from Aldrich
5. Hot plate
6. Ice bath
7. Vacuum rotary evaporator - Buchi Rotovapor R
8. Assorted volumetric glassware
9. 200 ml glass bottle

b. Reagents

1. Diethyl ether PQ, Burdick and Jackson
2. 95% Ethanol, AR
3. Diazald, Aldrich chemical
4. Potassium hydroxide, AR
5. 20% Sulfuric Acid, AR

c. Extraction

1. Twenty (20) g of feed was weighed into a 200 ml glass bottle followed by one hundred fifty (150) ml of ethyl ether, 5 ml of 20% H_2SO_4, and 5 ml of Ethanol.
2. Sample was blended with a Polytron homogenizer for 5 minutes.
3. The extract was filtered through a Buchner funnel. The Polytron blade and shaft, jar and filter cake were rinsed with a fresh 50 ml portion of ethyl ether.
4. The extract was transferred to a 200 ml volumetric flask and diluted to volume with diethyl ether. The contents of the flask were mixed thoroughly.
5. Group 3 extract; a 1 ml aliquot was transferred to a 5 ml volumetric flask and diluted to volume with diethyl ether.
   Group 4 extract; a 1 ml aliquot was transferred to a 50 ml volumetric flask and diluted to volume with diethyl ether.
   Groups 1 and 2 extracts required no further dilutions.

d. Methylation - performed under a hood - Diazomethane is toxic.

1. Diazomethane was prepared as directed on the Aldrich Diazald® bottle using the ethereal ethanolic method.
2. Ten (10) ml of ethereal diazomethane was added to 1 ml of extract prepared in c. 5. in a 250 ml flask. The flask was covered and allowed to stand overnight in a hood.
3. One (1) ml of toluene was added to each sample and the ether and excess diazomethane was evaporated using a vacuum rotary evaporator at 40°C, leaving the toluene behind.
4. The extract was transferred to a 200 ml volumetric flask with hexane and diluted to the mark with hexane.

e. Standard Preparation

1. An accurately weighed amount (100 mg) of Dicamba test material was transferred into a 100 ml volumetric flask and toluene was added to the mark to create a 1000 ug/ml standard.
2. A 0.5 ml portion of the 1000 ug/ml standard was diluted to 100 ml with toluene to create a 5.0 ug/ml standard.
3. One (1) ml of the 5.0 ug/ml standard was pipetted into a 250 ml flat bottom flask and the toluene was removed with a vacuum rotary evaporator at 50°C.
4. One (1) ml of a group 1 extract prepared as described in c. 5. was added.
5. The standard was methylated as described in d, then diluted to 100 ml in the final step to create a 0.05 ug/ml methylated standard.
6. A series of standards were prepared for the detector linearity check in the following manner; 0.5, 1.0, 2.0, 4.0, 5.0 and 6.0 ml of the 0.05 ug/ml standard prepared in step e. 5. were diluted to 10 ml with hexane to create 0.00250, 0.0050, 0.010, 0.020, 0.025 and 0.030 ug/ml standards, respectively.

u - Read as micro (10^{-6})

f. G. C. - Varian 2400 fitted with an electron capture detector.

1. Parameters

a. Column - 6' x 4 mm ID glass column packed with 10% DC-200 + 1.5% SP2401 on 80/100 mesh Gas Chrom Q.

b. Temperatures

1. Column - 170°C
2. Detector - 280°C
3. Injector - 250°C

c. Carrier flow (N_2) 35 ml/min.

2. Method

a. The linearity of detector response was determined by injecting each standard prepared in e. then plotting peak height vs. concentration. The correlation determined by linear regression was greater than 0.9.

b. The samples and the 0.025 ug/ml standard were injected as follows; sample, standard, sample, sample, standard, sample. The test article concentration in feed was calculated from this series of injections in the following manner:

$$ppm = \frac{\text{Pk Ht sample}}{\text{Pk Ht std.}} \times \text{Std. conc. (ug/ml)} \times \text{dilution factor} \times \frac{1}{\text{sample weight (}}$$

u - Read as micro (10^{-6})
Pk Ht - Peak height
Std. - Standard
Conc. - Concentration

V. STATISTICS

A. METHODS

1. Randomization Procedure

At the end of the pretest period, each animal number and the corresponding body weight was entered in a magnetic-disc data base which was used as the data source for the computer-calculated randomization procedure. The randomization procedure was carried out for all animals of the same sex as described below.

The mean body weight for all animals of the appropriate sex in the quarantine population was computed after which the absolute difference from the quarantine population mean weight was computed for each animal. The animals were then sorted in order of increasing absolute difference from the population mean. After the initial sorting, the appropriate number of blocks of animals was designated. The required number of animals was assigned to treatment groups by randomizing each successive block of animals. Bartlett's Chi-square test for homogeneity of variances was performed on these groups. If the group variances were judged to be non-homogeneous, new randomizations were generated until homogeneity was established at which time the randomization was accepted.

2. Data Analysis

Body weights and food consumption (weeks 1-13 and every 2 weeks thereafter), hematological, biochemical and urinalysis parameters (months 6, 12, 18 and 24) and absolute and relative organ weight data (12 month and termination) were analyzed using Bartlett's test for homogeneity of variance and analysis of variance (one way classification). Treatment groups were compared to the control group, by sex, using the appropriate t-statistic (equal or unequal variance), as described by Steel and Torrie[1] and Ostle[2]. Dunnett's[3] multiple comparison tables were used to determine significance. All statistical tests were two-tailed, with $p<0.05$ and $p<0.01$ used as levels of significance.

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Survival data and data on time to neoplastic lesion were analyzed using the computer program of Thomas, Breslow and Gart[4]. Statistical procedures included in this program are the Kaplan-Meier and standard methods for computing survival curves, Cox's test for linear trend in proportions, and both Cox's test and Gehan-Breslow's generalized Kruskal-Wallis test for comparing survival distributions. Data on time to neoplastic lesion were analyzed for all benign tumors, all malignant tumors, all tumors combined, and for each individual tumor type that appeared in two or more animals in the high dose group. When appropriate, the option for deleting early deaths was also used.

8. RESULTS

The results of the data analysis are discussed in the appropriate sections of the report.

Edwin I. Goldenthal, Ph.D.
Vice President and Director of Research
Study Director

2/27/85
Date

163-694

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VI. IN-LIFE PHASE

A. METHODS

1. Animal Acquisition and Maintenance

Three hundred sixteen male (316) and 304 female Charles River CD® rats (3 weeks old) were received from Charles River Breeding Laboratories, Inc., Portage, Michigan on June 15, 1981. The rats were observed twice daily during an approximate 2 week acclimation period. Two hundred forty (240) males (83-114 g) and 240 females (80-114 g) with no physical abnormalities were selected randomly (see section V.A.1. for randomization procedure) and assigned to groups as follows:

Group	Dosage Level (PPM)	Number of Rats	
		Male	Female
1	0	60	60
2	50	60	60
3	250	60	60
4	2500	60	60

All unassigned rats were removed from the study room.

The rats were housed individually in suspended wire-mesh cages in a temperature - (67-82°F) humidity - (32-78%) and light- (12 hour light/12 hour dark) controlled room. Water and control and treated diets were available ad libitum. The rats were ear tagged for individual identification. Ear tags were verified after the initial tagging, at the time of blood and urine collection, at each cage change and prior to necropsy.

The basal laboratory diet was Certified Rodent Chow® #5002 (Ralston Purina Company, St. Louis, Missouri). Analysis of each new lot of Certified Rodent Chow® #5002 was performed by Raltech Scientific Services, St. Louis, Missouri. The IRDC water supply is analyzed on a quarterly basis for the presence of heavy metals, pesticides and coliforms. The results of these diet and water analyses are retained in the Archives of IRDC and are available upon Sponsor request.

2. General Observations

a. Appearance and Behavior

The rats were observed twice daily for signs of overt toxicity, mortality and moribundity. These signs were recorded when noted. Detailed observations, including the incidence, first appearance, size, location and growth of palpable masses, were recorded weekly.

b. Mortality

Any rat showing signs of severe debility or toxicity, particularly when death appeared imminent, was sacrificed in extremis. Moribundity and mortality were recorded on the day noted.

c. Body Weights

Individual body weights were recorded twice during the pretest period, weekly for the first 13 weeks and once every 2 weeks thereafter.

d. Food Consumption

Individual food consumption values were recorded weekly for the first 13 weeks and once every 2 weeks thereafter. From these values and body weight values, compound consumptions were calculated.

VII. CLINICAL LABORATORY TESTS

A. METHODS

Laboratory tests were run on 10 rats/sex twice prior to study initiation (baseline 1 and 2) and on 10 rats/sex/group at 6, 12, 18 and 24 months of study.

The blood samples were obtained via puncture of the orbital sinus plasma from rats fasted overnight (approximately 18 hours). Urine samples were collected during this fasting period from rats housed individually in stainless steel metabolism cages.

1. Hematology

Hematological measurements were determined on whole blood and included total leukocyte count[1], erythrocyte count[1], hemoglobin[1], hematocrit[1], platelet count[1], reticulocyte count[2] and differential leukocyte count[2]. The hematological indices mean corpuscular volume (MCV)[1], mean corpuscular hemoglobin (MCH)[1], and mean corpuscular hemoglobin concentration (MCHC)[1] were automatically calculated by the analyzer (Ortho ELT-8).

2. Biochemistry

Biochemical measurements were determined on serum and included sodium[3], potassium[3], chloride[3], calcium[4], phosphorus[5], alkaline phosphatase[6], total bilirubin[7], aspartate aminotransferase (AST)[8], alanine aminotransferase (ALT)[9], lactic dehydrogenase (LDH)[10], urea nitrogen[11], creatinine[12], total protein[13], albumin[14], globulin (calculated), cholesterol[15] and glucose[16].

3. Urinalysis

Urinalysis determinations included color[17], appearance[17], microscopic examination of sediment[17], specific gravity[17], volume[17], pH[18], protein[18], glucose[18], occult blood[18], nitrites[18], bilirubin[18], ketones[18] and urobilinogen[18].

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VIII. <u>PATHOLOGY</u>

A. METHODS

1. Macroscopic

All animals received a complete post mortem examination under the direct supervision of a pathologist. All survivors at termination, all animals sacrificed <u>in extremis</u>, and all animals sacrificed at 12 months were euthanized by carbon dioxide asphyxiation.

After a thorough external examination, each animal was opened and the contents of the abdominal, thoracic and cranial cavities were examined both <u>in situ</u> and after removal and dissection. All macroscopic abnormalities were recorded on the Pathology Record sheet.

Representative samples of protocol-designated tissues were collected and placed in phosphate-buffered neutral formalin. A full complement of tissues was collected from all animals.

2. Organ Weights

Protocol designated tissues were trimmed free of fat and connective tissue and weighed. Tissue weights from all animals surviving until the scheduled terminal sacrifice as well as 10 per sex per group surviving until the scheduled 12 month sacrifice were recorded along with post mortem body weights. The following tissues were weighed:

Brain (with stem)	Liver
Heart	Ovary (2)*
Kidney (2)	Testis (2)

*Weighed after fixation

3. Microscopic

Representative samples of protocol designated tissues were processed for the preparation and microscopic examination of hematoxylin-and-eosin-stained paraffin sections. A full tissue complement was prepared for all animals sacrificed at 12 months, at study termination and all animals which died or were sacrificed <u>in extremis</u> during the course of the study. In addition, sections were prepared of the entire head

(included tongue, nasal cavity, turbinates, paranasal sinuses, nasopharynx, portions of the oral cavity and middle ear) from all animals.

A four-step grading system of trace, mild, moderate and severe was used to define gradable lesions for comparison between dosage groups. A complete listing of tissue accountability can be found in the Tissue Inventory (Appendix K).

The following list constitutes the full complement of tissues:

- Adrenal (2)
- Brain (3 levels: fore, mid and hind brain)
- Eye with contiguous Harderian gland (2)[a]
- Gastrointestinal tract:
  - esophagus
  - stomach
  - jejunum
  - colon
- Gonads:
  - ovary (2)
  - testis with epididymis (2)
- Heart
- Kidney (2)
- Liver (3 sections)
- Lung with mainstem bronchi[b] (all lobes)
- Lymph nodes:
  - mediastinal, mesenteric and regional when applicable[c]
- Mammary region[d]
- Pancreas
- Pituitary
- Prostate and seminal vesicle
- Salivary gland, mandibular with submandibular lymph node
- Sciatic nerve
- Skeletal muscle (thigh)
- Skin
- Spinal cord, cervical
- Spinal cord and vertebrae (lumbar)
- Spleen
- Sternum (bone and marrow)
- Thymic region
- Thyroid - parathyroid complex[e]
- Trachea
- Urinary bladder[f]
- Uterus (2 horns and cervix)
- All tissue masses
- All gross lesions

[a]Eyes were fixed in a glutaraldehyde fixative.
[b]Lungs were perfused with formalin through the trachea.
[c]When a tissue mass was present, the lymph node draining the region of the mass was examined.
[d]At times these tissues could not be identified with the unaided eye because of physiologic variation in size. However, tissue from the region was fixed for microscopic evaluation.
[e]Parathyroids could not always be identified macroscopically. They were examined microscopically if in the plane of section and in all cases where they were noted as grossly enlarged.
[f]Urinary bladder was perfused with formalin and examined after contracture.

END